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New data on the Role of Gamma Aminobutric Acid.

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Recent studies have focussed considerable attention on gamma-aminobutric acid (GABA). It was discovered in large amounts in mammalian brain by Roberts et al and others (I,2) and Awapra et al (3) in I950. The identity of this substance was proved by Udenfriend (4) in the same year. Widespread investigations of GABA action followed the establishment by Bazemore et al (5) and Hayashi et al (6) of its presence in "Factor I", and the discovery of its inhibitory effect on nervous activity. According to Mc-Lennan (7) Factor I contains substances other than GABA the action of which is not always identical with that ofFactor I . In another report the same author (8) comes to the conclusion that GABA is absent from brain containing Factor I. He postulates that GABA is a fragment of a larger molecule existing in Factor I to which he ascribes inhibitory activity. Inspite of this, GABA generally believed to play an important role in the accomplishment of inhibitory processes. Its effect on blood pressure, respiration and cardiac rythm has been ascribed to its periferic ganglion blocking action (9). Despite numerous investigations the true mechanism of GABA action on nervous processes is not yet known. Of special interest is its effect on the structure and behaviour of synaptic membranes and on those of neurones. GABA induces a repolatisation (or hyperpolarization) of cell membrane (IO). Koshtoyantz and Kokina (II) have shown that GABA and B-alanine have a similar effect on membrane potentials of organisms devoid of a nervous system, such as infusoria.

According to Boistel & Fatt, GABA excercises a similar effect as that observed on the stimulation of the inhibitory fibres of crustacean muscle. The increase in membrane conductance observed under the action of GABA and inhibitory fibre stimulation was interpreted as an increased permeability to Cl.(I2). chloride ions

Studies carried out by us in the course of a number of years have shown that the hypoglycaemic effect of insulin is not observed when it is given on the development of ~~cortical~~ the inhibition of the brain cortex obtained by extinguishing an already established conditioned insulin hypoglycaemia, (I3-I5). latent

Having in mind the role of GABA ~~in~~ inhibitory processes and its action on membrane structure, and on the other hand knowing that one of the main mechanisms underlying the hypoglycaemic effect of insulin is an enhanced membrane permeability to glucose (especially in muscle and fat tissue), it was interesting to study the effect of GABA on glucose transport in muscle tissue.

Experiments were made on isolated, intact rat diaphragm, which according to Kipnis and Cori (I6) and Randle and Smith (I7) is the most suitable object for the study of glucose permeability into muscle. The diaphragm was obtained from male rats of 80-I20 gr. wt. Incubation was carried out under anaerobic conditions at 37°for one hour in Krebs-Ringer bicarbonate buffer at pH-7.4. In our experiments it was shown that in this solution glucose uptake by rat diaghram was more pronounced than that observed in the bicarbonate buffer used by Randle (I8). ~~As described by Randle~~ Glucose was added to IOmls

of incubation medium to obtain a rinal concentration of 2.5mg /ml . After incubation its amount was determined colorimetrically by the Somogyi modification of Nelson's method. GABA was added to the medium to obtain concentrations of 0.5 to 50 µg/ml. and crystalline protamine zinc insulin to make IO^{-3} -0.I units /ml. The results obtained are presented inTable I.

Table I-Comparison of the effect of insulin (0. I unit /ml) and GABA (IO µg/ml) on uptake of glucose by isolated rat diaphragm under anaerobic conditions. (Glucose uptake given in mg. per I gr. of wet diaphragm).

Control	GABA IO µg/ml	Insulin 0,I unit/ml	Insulin I unit/ml. GABA IOmg.
6·37 ± 1·71 (11)*	21·40 ± 3·88 (14)	16·53 ± 3·78 (13)	28·1 ± 3·64 (11)

x The number of experiments are given in brackets.

The results obtained indicate that about more than three times increase in glucose uptake ensues rrom the addition of insulin(0.I unit/ml). This is further enhanced by the addition of GABA (IO µg/ml). The most pronounced glucose transport, however, takes place on the simultaneous addition of GABA and insulin, where glucose uptake by the diaphragm is increased above 2 times.

Being convinced that GABA in IOµg/ml concentrations has a greater effect on glucose uptake by muscle tissue than insulin, we tried the influence of various doses. The results obtained with 0.5-50,0 µg/ml amounts are given in table II.

Table II. The effect of decreasing concentrations of GABA on glucose uptake by rat diaphragm- expressed in mg. per I gr. of fresh tissue.

Control	50 µg/ml	IO µg/ml	5µg/ml	Iµg/ml	0,5 µg/ml
8·92 ± 1·24 (10)	4·9 ± 1·9 (3)	17·85 ± 1·22 (4)	22·7 ± 6·48 (6)	19·15 ± 4·70 (10)	12·63 ± 2·27 (6)

It is obvious that the effect of GABA on glucose uptake ~~does~~ is not ~~grow~~ enhanced with the increase of its concentration. On the contrary smaller doses seem to have a greater influence. Thus , for instance 5µg. seem to evoke a more pronounced glucose uptake than that produced by IO µg/ml. An uptake equal to IOµg. was obtained by I µg/ml. The considerable effect of GABA in such minimal doses is worthy of attention . Amounts as small as 0.5 µg/ml increases glucose uptake by about 1,5 times. From table II it is obvious that 50 µg of GABA excercise an ~~contrary~~ inhibitory effect ~~to that obtained from smaller doses (IO-Iv0 µg).~~ Here ~~in~~ of glucose uptake of decrease about two foids ~~is noticed.~~

Knowing that insulin produces its effect in concentrations of ~~I0~~ 1/1000 of a unit/ml (I8) and that its amount in serum or normal animals and humans is about ~~I0~~ 1/10000 unit/ml (I9), the effect or such low concentrations or both insulin and GABA (0.5-I µg/ml) were tested. It was noticed that I µg of GABA greatly enhanced glucose transport, while 0.5µg have an effect almost equal to that of ~~I0~~ 1/1000 of a unit of insulin

In this series of experiments the amount of glycogen in diaphragm was also determined by the method of Morris (20). The results obtained are given in table III.

Table III. Glycogen content of rat diaphragm in mg. per I gr. of wet tissue.

Control	Insulin O,I unit/ml	GABA IO/ml	GABA-Insulin IOμg/ml-O,I unit/m
0·8 ± 0·44 (5)	2·12 ± 0·60 (6)	2·22 ± 0·46 (4)	2·5 ± 0·05 (3)

The data obtained indicate that insulin and GABA considerably increase glycogen content of diaphragm, .

A noticeable rise in the amount of glycogen takes place even in 1 μg/ml of GABA. These results are compatible with those obtained while studying glucose uptake by muscle tissue from the incubation medium.

Muscle glycogen was also determined histochemically by the method of Bawer. As shown in fig. I-3, a greater accumulation of glycogen in muscle tissue takes place in the presence of GABA (IO μg/ml) than onthe addition of insulin (O.I unit/ml).

A similar increase in glucose uptake by muscular tissue was noticed on the administration of GABA into the femoral artery under nembutal anaesthesia.

Blood samples for the determination of glucose were taken simultaneously from the femoral artery and vein. It was shown that the A-V difference is considerably increased from I-IO minutes following its injection.

According to Soskin and Levin (2I) Geiger et

al (22) and Park et al (23) insulin does not affect glucose uptake by the brain. A few preliminary experiments seem to point to an immediate effect of insulin on brain glucose uptake, namely within 2-5 minutes following its intracarotid administration, prior to the development of its hypoglycaemic effect . From this point of view it was interesting to study the effect of GABA on this process. Experiments were carried out on previously operated dogs where a carotid loop was obtained and the branches of the external jugular vein, all except the posterior facial, were ligatured. In this way blood taken from this vein came directly from the longitudinal sinus of the brain. Blood samples for the determination of glucose were taken from the carotid artery and jugular vein following the injection of2.0-2.5 µg. of GABA into the artery. Table IV presents the results obtained.

Table IV- The Effect of GABA on brain glucose uptake.A-V difference in mg. %.

Prior to GABA injection	After GABA injection		
	2m.	6m.	10m.
5·37 ± 4·82 (8)	13·87 ± 2·94(8)	4·87 ± 3·66(8)	4·12 ± 5·06 (8)

The results obtained indicate that on the 2nd minute following GABA administration brain glucose uptake considerably increases until it gradually reaches its previous amount. This temporary increase in brain glucose uptake is, most probably, due to its rapid distribution throughout

the body, (except the brain) and its rapid excretion (24).

Data concerning the permeability of GABA into the brain are contradictory , Marrazzi et al. (25) and Hayashi et al (6) report an inhibitory effect on central synaptic transmission following the injection of GABA into the carotid artery, According to van-Gelder and Elliott (24), no increase in the content of GABA is observed in brain following its intro-peritoneal or intravenous administrations. Elliott and Jasper (26) find that GABA passes from the blood into the brain very sparingly. Others claim that it passes only in areas of local destruction or the blood brain barrier (27). At present the enhanced permeability of brain towards glucose caused by the administration of GABA cannot be explained . However, the immediate effect of GABA on the blood-brain barrier , as well as its effect on brain glucose metabolism and in this way the promotion of its uptake by brain cannot be excluded. Experiments dealing with the mechanism of glucose transport under the action of GABA are now in progress in our laboratory.

In connection with these experiments it was interesting to study the effect of β alanine on glucose uptake by rat diaphragm. The experiments undertaken were similar to those described above.

The results obtained are presented in table V

Table V -The Effect of B-alanine on glucose uptake by rat diaphragm, mg.per 9m. of wet tissue.

Control	B-alanine 10 µg/ml	B-alanine 20 µg/ml
8.07 ± 2.01 (7)	2 57 ± 2.06 (7)	3.9 ± 0.95 (2)

From data presented it is obvious that B-alanine in amounts of IO-20 µg/ml. considerably inhibits ~~in~~ glucose uptake by muscle . In this respect it was interesting to study the effect of B-alanine on the promotion of glucose uptake induced by GABA and insulin. The results obtained are presented in tables VI & VII.

Table Vi - The Effect of B-alanine on glucose uptake of rat diaphragm in the presence of GABA in mg. per gm. of wet tissue.

Control	GABA Iµg/ml.	GABA-B-alanine 50µg/ml 20µg/ml	GABA 20µg/ml Balnine 3µg/ml	GABA Iµg/ml Bal. 10µg/ml	GABA 1µg Bal. 20µg/ml
9.0	I9,6	II,8	4.2	4.2	5.8

Table VII- The Effect of B-alanine on glucose uptake of rat diaphragm in the presence of insulin in mg. per gm. of wet tissue.

Control	Insulin O.Iunit/ml	B-alanine IOµg/ml ins.O.Iunit/ml	B-alanine 10µg ins.O.Iunit/ml	B-alanine 10µg/ml ins.IO^{-3} unit/ml
8·86±1·32 (3)	19·73±1·28 (3)	16·93±0·85 (3)	19.05±0.95 (2)	14 (1)

From the table VI and VII it is obvious that B-alanine considerably inhibits the effect of GABA on glucose transport, while the action of insulin remains almost unchanged.

A number of investigators have demonstrated that large concentrations of ω - amino acids (C_2-C_5) have an inhibitory effect on nervous activity similar to GABA. However, there are some indications that the previous administrations of β-alanine inhibit the effect of GABA (28). In this respect the results obtained by us in regard to β-alanine are of some interest. It may be concluded that the action of β-alanine on the effect ofGABA is of a competetive nature.

Histochemical studies have shown that insulin and GABA increase the content of the basal substance of the connec tive tissue enveloping the muscle fiber_____ i.e-the acid mucopolysaccharides. These have been detected through their metachromatic staining property with toliudine blue and also by the method of Halle (29), based on the affinity ofthe acid mucopolysaccharides to absorb colloidal iron. The sections are subsequently embedded in potassium ferrocyanide and Prussian blue is formed at the sites of acid mucopolysaccharides. Insulin and GABA seem to produce depolimerization and swell ing of acid mucopolysaccharides. Such changes inthe proper ties of the main substance ofconnective tissue may partly account for the increase in muscle permeability to glucose. This problem, however, needs further study.

The following question arises; Has GABA any role in the transport of glucose or any other substance in the organism? The results of our experiments seem to be in accord with such an idea. GABA in minimal amounts such as 0.5 μg/ml. induce a marked increase in glucose uptake by muscle and in

its glycogen content. With the exception of the brain, other organs contain very small amounts of GABA (from 0.2-IO µg/gr. of tissue) (30). These small amounts, however , according to our experiments, seem to have a considerable effect on glucose uptake. The question, whether insulin may produce its effect on glucose transport in muscle through the enhancement of GABA formation remains to be answered. Our experiments demonstrate the inhibitory effect of β-alanine on the increased glucose uptake produced by GABA. Such an effect of β-alnine was not observed in respect to insulin. In this connection the action of GABA and insulin on glucose uptake by brain is also of interest. As mentioned above, GABA enhanced the penetration of glucose into brain after 2 min. following its injection, insulin seems to have a similar effect. It seems likely that glucose uptake by muscle through different mechanisms. The final elucidation or this problem, however, remains to be given.

The conclusion may be drown that insulin and GABA increase glucose uptake by muscle through different mechanisms.

According to a number of investigators, as for instance, Gravioto et al. (3I), Dawson (32) and van-Gelder and Elliot (33) in insulin hypoglycaemia, the amount of GABA in brain is decreased. This is obviously due to the fact that during insulin hypoglycaemia brain consumes almost no glucose, thus the formation of GABA by way of glutamic acid cannot take place.

It is generally accepted that insulin does not affect the glucose metabolism of brain . However, in muscle tissue, by promoting glucose transport and oxidation, it increases the formation of glutamic acid through the tri-

carboxylic acid cycle . The main route of GABA formation is through the decarboxylation of glutamic acid by the action of the corresponding decarboxylase, the activity of which is very high in brain and very insignificant in other tissues. On the other hand, the extensive distribution of GABA indicates that its formation in other ways cannot be excluded.

The results obtained by us, together with data from the literature, indicate that GABA has a widespread effect in animal organisms and that its function cannot be restricted to that of an inhibitory transmitter. McKhann and Tower (34) question of the role of GABA as a factor taking part in the mediation of innibitory impulse. They find that its presence in brain in large quantities and its high turnover-rate are not indicative of its role as a neuro-humor. They suggest that GABA affects brain function by participating in its energetic metabolism.

The question concernig the neuro-humoral action of GABA lies out of the scope of this report, ~~however according to many authors there seems to be no doubt about its significance as a transmitter of inhibitory processes.~~ But it is evident that the role of GABA in the organism cannot be confined to this. It may participate in the energetic metabolism of the brain not only by way of the tricarboxylic acid cycle but also through the promotion of glucose penetration into nerve cells. The results of our experiments seem to point to a possible role of GABA in the metabolism of organs besides the brain and raise new questions concerning this substance which is widely distributed in plants and microorganisms as well.

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Brain Glucose Uptake During Its Various Functional States.

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(Based on the investigations of the author and coworkers- Yeghian V.B., Khachatrian G.S., Mkheian E.K., Karageosian C.K., Kechek G.A. and Urgandjian M.G.)

Many questions concerning the biochemical processes underlying the different functional states of the brain require further study. The investigation of the functional biochemistry of the brain involves many difficulties. The experimentator in this field should set up such conditions which would not disturb the normal functioning of the brain. Even in the most careful of in vitro experiments, brain tissue cannot be undergoing some changes, on the other hand, present in vivo methods are not such as to permit the study of the more profound processes inherent in excitory and inhibitory states. Moreover, the biochemist, studying the interrelationship between cerebral metabolism and function, has to use some physiological manifestations as criteria for the development and duration of the excitatory and inhibitory processes. Besides this, attention should be paid to the specific properties of the stimulating agent used, as well as to its effect on brain metabolism, since not all stimulants and narcotics have similar effects on brain metabolism. That brain processes very poor energetic sources despite the great energy its activity demands is a fact which should also be borne in mind.

The energy required by the brain is provided mainly by glucose, brought by the blood stream. Consequently, in studying the biochemistry of cerebral function, the interrelationship between cerebral metabolism and metabolic processes in effector organs, has to be studied, too. The brain, in accomplishing the finer regulation of metabolic processes, provides for its own activity. Certain functional states of effector organs influence cerebral metabolism, and function. The above mentioned problems are not all that are met with in the study of the functional biochemistry of the brain, but they are enough to show the many difficulties that are encountered during the study of this problem and also to account for the controversial results obtained by various investigators in this field.

Many authors, in studying brain metabolism during excitation and inhibition, various workers produce these functional states through the use of pharmacological agents or electrical stimulation. These, of course, are not adequate methods for obtaining a true picture of normal cerebral function. One of the most suitable methods for studying this problem is that of conditioned reflexes. Pavlov and other investigators have shown, that the development of conditioned reflexes is due to the excitation of certain cortical centres from which excitatory processes can irradiate to other cortical and subcortical centres. It has also been established that the systematic daily administrations of the conditioned stimulus alone, conduce to the fading away of the conditioned reflex with development of inhibitory processes in the corresponding cortical centres. The inhibitory processes can

irradiate to other cortical and subcortical areas.

Numerous studies emphasize the increased utilization of glucose and oxygen during hightened cerebral activity. As shown by the investigations of Geiger , and others the transport of glucose into brain is not a simple process of diffusion. The amount of glucose in brain does not always agree with its concentration in blood. According to Wolf, glucose administered into the cerebrospinal fluid is not taken up by brain tissue. The mechanisms of glucose transport into brain is not, as yet, very well understood. Most probably, the transport of glucose into the brain depends on the metabolic processes of brain tissue itself, and as well as the functional state of the blood brain barrier. A number of authors have shown, that insulin does not affect this process. Geiger, demonstrated by perfusion experiments (of the brain) the importance of the liver in the transport of glucose into brain. It was shown that brain began to take up glucose from the perfusion fluid only, following its passage through the liver, or upon the addition of cytidin and urydin to the perfusion fluid. The investigations of a number of authors, as well as our own indicate that during narcotisation, in spite of some increase in blood glucose level the uptake of the latter by brain tissue is reduced. In alert and excited states, on the contrary, sugar uptake by brain tissue in enhanced, . Some stimulating agents, however, produce the opposite effect. These and many other studies indicate, that a complicated mechanism underlies the uptake of glucose by brain tissue, which is considerably affected by the functional state of the brain and the metabolic processes in effector organs.

In our laboratory, we have studied the problem of glucose uptake by cerebral tissue, during different functional states of the brain. The experiments were carried out on dogs. Blood samples were taken from a carotid loop and from the jugular vein all the branches of which with the exception of the posterior facial, had been previously ligatured. Thus the blood obtained came directly from brain.

FIG I.

Fig I is a scheme of the operation. The same ingredients were determined both in arterial and venous blood. The difference noted gave the uptake of those substances by brain tissue. Blood flow rate was also determined. Conditioned reflexes have been developed in respect to the following stimuli - The administration of food, rich in sugar(sugar loading) epinephrine and the electrical stimulation of the skin causing pain.

The arterio-venous (A-V) difference in regard to the ingredients studied, was determined during the effect of unconditioned and conditioned stimuli, as well as the development of cortical inhibition. The latter was obtained through the method of extinguishing an already established conditioned reflex by the repeated, daily administrations of the conditioned stimulus alone.

The first series of experiments were carried out on five dogs. Here, the unconditioned stimulus used was sugar loading. The animals were given 50-120 gr. of sugar mixed with powdered meat. This amount was divided into 3 portions given at intervals of 5 minutes. The administration of food was conjugated with the sounding of a buzzer. Blood glucose and pyruvate levels

were followed for 90 minutes , during which samples were taken at intervals of 30 minutes. The above mentioned amounts of sugar lead to an increase in blood glucose and pyruvate levels . Similar results were obtained by the conditioned stimulus alone, that is to say in cortical excitation. Under the existing circumstances the uptake of glucose and pyruvate by brain tissue was increased considerably. By way of illustration the results obtained on one dog will be mentioned . Similar changes have been obtained on the other four dogs also. As shown in Fig 2, the conditioned increase in blood glucose and pyruvate levels.

FIG. 2.

is accompanied by a corresponding rise in their consumption by brain tissue. The same effect was noticed on the first three administrations of the conditioned stimulus. Its further trials, however, led to the fading away of the conditioned reflex with the development of cortical inhibition, during which the above mentioned changes were not to be seen. With the more profound establishment of cortical inhibition, the dog did not respond to the conditioned stimuli any longer, and was often in a drowsy state. During this period, a reversal of effects waas observed. As

FIG 3.

shown in fig 3, blood glucose level, as well as its uptake by brain tissue were considerably reduced.

Fig 4, presents a similar picture regarding pyruvic acid, and its utilization by brain.

FIG 4.

As to the uptake of pyruvate by brain contradic-

tory results have been reported. Our investigations, however indicate that on the whole brain consumes pyruvic acid.

In these studies the rate of cerebral blood flow has been determined through the use of radioactive phosphorus. During alimentary and conditioned alimentary excitation, circulation time is equal to 10 seconds; in cortical inhibition, it increases to 15 seconds. Thus, in cortical excitation, inspite of the higher rate of blood circulation, brain utilises more glucose and pyruvate. A reversal of effects is observed during the development of cortical inhibition.

The results of these investigations confirm our previous observations concerning the reversal of metabolic processes during cortical excitation and inhibition. On the establishment of a conditioned reflex towards sugar loading cortical excitation is accompanied by a rise in blood glucose and pyruvate levels together with their increased uptake by brain tissue. Quite the reverse is seen on the development of cortical inhibition.

It was interesting to see whether a similar relationship in regard to blood glucose and pyruvate would be noticed in epinephrine hyperglycaemia. On studying this question we found out that in epinephrine hyperglycaemia the uptake of glucose by brain is enhanced.

FIG 5.

While in the case of hyperglycaemia, produced by narcotization the consumption of glucose by brain is , on the contrary, reduced. As will be seen later, a similar effect is obtained during the

electrical pain causing stimulation of the skin. Thus it was confirmed that the uptake of glucose by brain tissue does not always depend on its amount in blood.

In this respect the functional state of the brain and the specific effects of the stimulating agent itself on brain function , are of definite importance.

As was indicated above, on the development of the inhibitory process in respect to conditioned alimentary hyperglycaemia, the level of blood glucose substantially decreases, with a corresponding fall in brain glucose uptake. This shows that in this case cortical inhibition leads to the increased secretion of insulin. With the purpose of confirming this suggestion insulin was administered to experimental animals . It was observed that during insulin hypoglycaemia a similar depression in brain glucose uptake was noticed. These results are in accordance with those reported by Somogyi. The results obtained by sugar loading are indicative of the activation of the insular system during the development of cortical inhibition. It must be noted that on the development of cortical inhibition blood sugar is usually at a remarkably low level and brain uptake is very much depressed. As shown in Table I, within the first few days of this state the administrations of 120 gr. of sugar increase neither blood glucose level, nor glucose uptake by brain tissue.

Table I.

On the first day of sugar administration, the amount of glucose in arterial blood varies between 37-51mg. and in venous blood between 40-50 mg. %. , with no substantial A-V difference. On the following two administrations a gradual

rise in blood glucose level and brain consumption are noticed On the 2nd and 3rd administrations blood glucose level increases only by 12-15 mg.%, while the 4th administration evokes a rise of 30 mg. % i.e. an increase equal to that obtained prier to the inhibitory state. Brain glucose uptake is also increased to about 11-14 mg. %.

These and other results obtained during our investigations indicate the important influence of cortical impulses on the effect of a number of unconditioned stimuli.

The A-V difference in glucose was studied also following the electrical pain causing stimulation of the skin. Blood samples were taken prior to the application of the stimulus, as well as 5 and 20 minutes following its application. After the experimental animals became accustumed to the conditions of the experiment control tests were carried out with the purpose of determining the normal blood sugar level and glucose uptake of the animal in question.
Fig 5 represents the results of such experiments on one dog.

FIG 5.

As shown in this figure, the A-V difference of glucose in this animal varies between 6-8 mg. %. On the administration of the unconditioned and conditioned stimuli, inspite of some increase in blood glucose level, the A-V difference is considerably reduced and sometimes there appears even a negative difference. During the development of inhibition the uptake of glucose is again increased.

Assuming that hexokinase may have some effect on the transport of glucose into brain, it was decided to follow its activity in rat brain in experiments with sugar loading. It was interesting to follow hexokinase activity during alimentary and conditioned alimentary hyperglycaemia when brain glucose uptake was increased and during cortical inhibition when it was depressed. In these experiments the different fractions of glycogen were also determined. The above mentioned functional states were developed in experimental rats through the use of a special system, after which they were fixed in liquid oxygen.

The results of these experiments indicate that brain hexokinase activity is increased during alimentary and conditioned alimentary hyperglycaemia when an enhanced consumption by brain tissue is observed. On the development of cortical inhibition, hexokinase activity falls to about one half its control level. These data are illustrated in Fig 6.

FIG 6.

A certain correlation seems to exist between brain hexokinase activity and glucose consumption by brain. However, the permeability of glucose into brain does not seem to depend on hexokinase activity. This is confirmed by the investigations of Stone Klein who demonstrated that fructose penetrates into the brain with difficulty, in spite of the fact, demonstrated by Cori, that it is very easily phosphorylated in brain tissue. Glucose, as compared with other sugars, penetrates into the brain with great ease. Here it is metabolized very quickly, thus promoting the penetration of fresh amounts.

In those investigations, where the different fractions of glycogen—protein bound, lipoid bound and free—have been determined, it was observed that during alimentary hyperglycaemia the amount of total glycogen, is increased. The most noticeable rise takes place in respect to free glycogen, where an increase of 16-24 mg. % is observed. In conditioned alimentary hyperglycaemia the amount of total glycogen is somewhat increased with a corresponding decrease in protein bound glycogen. The quantity of free glycogen and of that bound with lipoids remains high. The results obtained on the development of cortical inhibition are of some interest. As shown in fig 7 the amount of total glycogen does not undergo any appreciable

FIG 7.

change while a considerable rise is noticed in the amount of free glycogen at the expence of protein bound glycogen.

A number of investigators have shown that in brain tissue free glycogen has the highest turn-over rate, and its amount undergoes considerable changes during the various functional states of the brain. Our findings are compatible with these data. Of special interest is the fact that during the inhibition of conditioned alimentary hyperglycaemia when the uptake of glucose by brain is reduced, the amount of free glycogen which is the most labile is increased. At the same time the amount of protein bound glycogen is decreased. From this it may be inferred that in cases of reduced glucose uptake free glycogen provides for the energetic demands of the brain.

In the course of our investigations an intense

diminution in brain glucose uptake, has often been observed, as for instance during the inhibition of conditioned alimentary hyperglycaemia, insulin hypoglycaemia and in the various stages of electrical, pain causing, stimulation of the skin and the correspondind conditioned reflex. A similar diminution in glucose consumption was observed following the extirpation of the superior cervical sympathetic ganglion.

These findings are of interest in connection with the investigations of Hasratian, who on removal of this ganglion in dogs noticed a distortion in normal conditioned reflexive function.

In the course of our experiments particularly on the development of cortical inhibition we have frequently met a negative A-V glucose difference. This effect, which was noticed even when glucose was determined chromatographically, does not seem to be connected with brain circulation rate, or the concentration of glucose in arterial blood. A negative A-V glucose difference has been reported by Batrak, Zakharov, and others. It has been obtained by us in experiments following narcotization, as well as in cases of fully maintained brain activity.

This raises the question as to what substance replaces glucose in such cases and provides the energy necessary for brain function.

As far back as 1930, Gerard had reported that during nervous activity substances other than carbo-hydrates are used. According to Mullins glucose is not utilized during nervous activity. Gronoll and Davies have shown that in

diminution in brain glucose uptake, has often been observed, as for instance during the inhibition of conditioned alimentary hyperglycaemia, insulin hypoglycaemia and in the various stages of electrical, pain causing, stimulation of the skin and the corresponding conditioned reflex. A similar diminution in glucose consumption was observed following the extirpation of the superior cervical sympathetic ganglion.

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the absence of glucose, the electrical activity of certain areas of brain and their oxygen intake are maintained for two hours. The perfusion of experiments of Geiger on cat brain in site are quite interesting. They demonstrate that in conditions of adequate aeration, in the absence of glucose in the perfusion fluid, brain activity is maintained for over one hour, in spite of the fact that glucose disappears during the first 10-15 minutes. In such conditions the respiratory quotient of the brain falls to 0.58, the amount of phospholipids and nucleic acids in brain cortex is reduced and there is an output of free amino acids from the brain. On the basis of experiments carried out with uniformly labelled (C14) glucose Geiger came to the conclusion that only 30-35 % of the glucose present in brain, is completely oxidized. During nervous activity, the quantity of glucose undergoing complete oxidation is even less than this. A considerable amount of glucose is spent in the synthesis of amino acids, proteins, lipids and other non-acid soluble substances which may be used in cerebral function. In cases of hightened brain activity, as for instance under the effect of metrasol, the break-down of phospholipids and galactolipids, is enhanced.

Although, normal functioning conditions for the organism are not maintained even in the experiments of Geiger, nevertheless compared with studies carried out on isolated ~~out on isolated~~ brain preparations, this is a significant step forward and the results obtained deserve due consideration. These results indicate the involvment of many substances in the realization of brain function.

In view of the fact that the electrical pain causing stimulation of the skin induces a considerable decrease in brain glucose uptake, together with observations reported in the literature concerning the ability of the brain to utilize its own constituents for energy requirements, we decided to follow the A-V difference of substances besides glucose. According to many authors, glutamic acid can serve as a source of energy for brain tissue. Recently its ability to penetrate the brain has been demonstrated. Welsh has shown that in mature animals, despite the constancy in the amount of brain glutamic acid, it is continuously being exchanged by that present in blood. Glutamine penetrates the brain with greater ease.

Experiments carried out in this connection demonstrate that during the conditioned reflex obtained on the electrical pain causing stimulation of the skin, the amount of glutamic acid increases in venous blood, and remains unchanged i arterial blood which indicates its increased out-flow from the brain. The results of such experiments, illustrated in fig 8, demonstrate that during the development of cortical inhibition on the fifth minute following the conditioned stimulus, brain consumes glutamic acid. Similar results have been obtained in respect to aspartic acid. As far as glutamine is concerned it is consumed by brain in control experiments.

In the course of the stimulation of the skin and the conditioned reflex following it glutamine is given out from brain, while on the development of cortical inhibition, it is again consumed. These results are illustrated in fig. 9

FIG 9.

In connection with the changes of the glutamic acid and glutamine, it was interesting to study the outflow of ammonium from brain. As shown in fig IO , in control experiments and in blood samples taken prior to stimulation, brain consume ammonium. FIG 10. On the electrical pain causing stimulation of the skin and the conditioned reflex following , this effect is reversed and an appreciable output of ammonium from the brain takes place. During cortical inhibition the production of ammonium by brain is continued and some consumption is noticed only on the 20th minute after conditioned stimulation.

In our experiments we have also determined the A-V difference in respect to glutathione , which is found in brain tissue in considerable amounts. It has been shown that during the electrical stimulation of the brain and the conditioned reflex developed in consequence of its repeated administrations glutathione is increased in venous blood, which indicates its outflow from the brain tissue. A reversed effect is observed on the development of cortical inhibition. The results obtained lead to the conclusion that during the electrical pain causing stimulation of the skin and the ensuing conditioned reflex, the depression in glucose uptake by brain is accompanied by an increased output of glutamic and aspartic acids, glutamine, ammonium and glutathione. These results and those of Geiger concerning the increased output of amino acids from the perfused brain in the absence of glucose, allow us to confirm the fact that

protein serves as a source of energy for brain, especially in cases of reduced glucose uptake.

When the glucose uptake by brain is reduced an increased consumption of pyruvic acid is also observed. This may indicate its usage by brain tissue under these conditions.

In cases of lowered glucose utilization, brain glycogen may also be of some importance as a source of energy, especially its free fraction, the amount of which increases at the expence of the protein bound form.

Another constituent of brain, acetylaspartic acid, seems also to take part in brain function. Its amount decreases following coffein stimulation, and prolonged excitation produced by four hour swimming. Preliminary experiments seem to indicate that it participates in the synthesis of acetyl choline.

The estimation of the A-V difference of phospholipids and cholesterol shows that these constituents also seem to take a certain part in brain function. Data presented in fig II show that during control experiments there

FIG II.

is a small output of phospholipids from brain, which is considerably increased during the electrical pain causing stimulation of the skin and the conditioned reflex developed. The opposite effect is observed during cortical inhibition. These results are in accordance with those of Geiger who found out a considerable decrease in the amount of phospholipids in brain cortex during cerebral activity , especially in the

absence of glucose in the perfusion fluid.

Fig. 12.

A similar pattern is observed in respect to cholesterol. Here also an increased output is to be seen after the electrical stimulation of the pain and conditioned stimulation. Inasmuch as the sensation of pain and emotional states are to a certain extent similar to each other, the possibility arises that in frequent emotional states and in stress the brain may produce increased amounts of cholesterol and this may atherosclerosis. If atherosclerosis are likely to develop. As shown in the figure, on the development of cortical inhibition there is, on the contrary, a consumption of cholesterol. Our investigations indicate also that various constituents of the brain are involved in its activity, in the case of lower glucose uptake in particular.

From the results of our experiments it may be concluded that during cortical inhibition there is a reversal of metabolic processes in brain tissue, which enables the synthesis of the substances utilized during its activity.

The Cortical Regulation of Carbohydrate Metabolism.

H. Ch. Buniatian.

(Based on the investigations of the author and coworkers. Yeghian V. B. , Aduntz G. Th., Hovhannissian A.S., Yessaian N.A., Khachatrian G.S., Mkneian E.Y., Karageosian C.G., Urgandjian M.G., Oydjian C. Movsesian C. G. and Hagopian J.

One of the important problems of biochemistry is the study of the cortical regulation of metabolic processes in effector organs, as well as the study of the biochemical processes underlying cortical excitation and inhibition. A number of investigations have been undertaken in our laboratory to study this problem.

In the present report, I shall give the results obtained concerning the effect of cortical activity on metabolic processes in effector organs. Usually, different functional states of the brain are obtained by the administration of stimulating and inhibiting agents. In our experiments we have abstained from the use of such means, knowing that these agents are not free from some side effects of their own.

The investigation of the higher centres of the brain is best carried out through the use of the method of conditioned reflexes, which was introduced by Pavlov. This method is undoubtedly by far the most adequate and physiological means for studying the various functional states of the cortical centres. Conditioned reflexes, being acquired, represent a

more perfect form pf adaptation to changes in the surroundings. They develop in association with an unconditioned, inborn reflex. When a stimulus, which in itself is absolutely neutral, is repeatedly applied simultaneously with an unconditioned stimulus, the two corresponding cortical centres are activated at the same time and a temporary nervous association develops between the two. Thus, the hitherto neutral stimulus acquires stimulative properties, and is transformed into a conditioned stimulus, the application of which evokes the same changes as the unconditioned stimulus. In this respect, the changes that are brought about in the organism by conditioned reflexes may be regarded as the result of cortical activity.

Pavlov discovered further a functional state of the brain cortex, which he named conditioned or internal inhibition. It may be also called cortical inhibition. Unlike the inborn inhibitory processes of the central nervous system, cortical inhibition is acquired and therefore of a temporary character. One of the methods used by Pavlov for developing cortical inhibition was the extinction of an already established conditioned reflex, which is attained through the repeated administrations of the conditioned stimulus itself.

In our investigations, in studying the cortical regulation of metabolic processes in effector organs, we have attained activated and inhibited states of the cerebral cortex by developing conditioned reflexes and then extinguishing them through the daily administrations of the conditioned stimulus. The unconditioned stimuli used in these studies were the intravenous injections of epinephrine and insulin, the administration

of food rich in sugar and the electrical (pain causing) stimulation of the skin. In cases where the unconditioned stimulus consisted in injecting a substance, the conditioned stimulus used was the act of the injection itself, where a neutral substance, such as an isotonic solution of sodium chloride, was used. In the other experiments, the conditioned stimulus was the sounding of a buzzer. During these studies, the following ingredients have been determined:- blood glucose, pyruvate, lactate, inorganic phosphorus, catechol amines, histamine, glutathione, glutamic and aspartic acids, glutamine and ammonium , as well as the various components of the blood clotting system. Changes in renal function have also been followed.

After a number of simultaneous daily administrations of the unconditioned and conditioned stimuli when the conditioned stimulus alone was applied, it called forth, as was expected, the same changes as those brought about on the administration of the unconditioned stimulus. This proved that the changes concerned were brought about by cortical activity. Then followed the extinction of the reflex, during which period a gradual fading of the changes studied was noticed. On continuing the administration of the conditioned stimuli, with the purpose of developing cortical inhibition, there came a time when not only no changes were observed regarding the ingredients in question, but even a reversal of the effects was noticed.

The results of our investigations clearly indicate that during the development of cortical inhibition, the metabolic processes in the effector organs go in the opposite direction, as compared with those obtained during cortical activity.

To illustrate this fact numerous examples may be produced from our experiments. In fig. I, for instance, we see

FIG I.

the effect of epinephrine and that of the conditioned stimulus , on blood glucose level. Epinephrine , given in amounts of 0.8 mgs., brings about a noticeable rise in blood-sugar level. After about ten trials, the injection of an altogether neutral substance such as saline solution, brings about the same increase in blood glucose as was brought about by epinephrine . This effect, however , gradually fades away and after the third application is no more noticed. On subsequent administrations of the conditioned stimulus alone, a state of internal inhibition develops gradually and a reversed effect is noticed, that is to say hypoglycaemia is obtained. What is more striking, epinephrine , administered during such a state, no more exercises its usual effect; it may even cause a slight fall in blood glucose level.

Similar results are obtained concerning inorganic phosphorus during conditioned insulin stimulation. As shown in fig 2., where the results are expressed as percentages of the initial values,

FIG 2.

insulin causes a decrease in the amount of inorganic phosphorus in blood. Even a greater fall is noticed on the administration of the conditioned stimulus.

Subsequent administrations of the conditioned stimulus lead to the establishment of cortical inhibition, during which period a considerable rise of the amount of blood phos-

phorus is obtained. Insulin, given under such conditions, evokes almost no change in the level of blood phosphorus. Similar results have been obtained in respect to blood glucose level.

The same thing is noticed in those experiments where blood glucose and pyruvate are determined following the administration of food rich in sugar conjugated with the sounding of a buzzer. The rise in blood-sugar and pyruvate, noticed on the administrations of the unconditioned and conditioned stimuli, is no more obtained after the third administration of the conditioned stimulus. As shown in fig 3, during this and the following three experiments the development of internal inhibition causes a further lowering of blood pyruvate level. These data, together with many others obtained during our experiments, indicate that cortical stimulation and inhibition have contrary effects on the metabolic processes of effector organs.

Pavlov pointed out the active nature of cortical inhibition and stated that it required much further study. The results of our investigations show that during cortical inhibition active processes are at work, the study of which gives us a means for following the development of cortical inhibition beyond the zero effect, when the administrations of the conditioned stimulus induce no change. It is established that inhibitory processes protect the nerve cells from complete exhaustion and enable its recovery. As will be reported in greater detail in another paper , studies at our laboratory have shown that during cortical inhibition there is a reversal of metabolic processes

even in brain tissue itself.

Here the reversal of metabolic processes in effector organs has been demonstrated. Thus inhibition embraces the whole of the organism. It undoubtedly serves the replenishment of those substances that have been used up during the heightened activity of the organ in question, subsequent to cortical excitation.

Now what is the mechanism of the reversal of the metabolic processes observed during cortical inhibition? The results obtained in the course of our investigations indicate that during the inhibition of certain cortical centres, the activity of the reciprocal system is increased. This is obvious from the facts obtained during the development of cortical inhibition in respect to conditioned epinephrine hyperglycaemia and conditioned insulin hypoglycaemia.

FIG 4.

As shown in fig 4, the conditioned stimulus produces the same increase in blood glucose level as epinephrine. On developing cortical inhibition, however, the same conditioned stimulus brings about a lowering of blood glucose level. The few subsequent administrations of epinephrine do not produce any hyperglycaemia. This is indicative of a depression in adrenal activity with a corresponding increase in insulin secretion.

A reversed picture is noticed on the development of cortical inhibition regarding conditioned insulin hypoglycaemia. Here a depression of insular activity is accompanied with an increase in adrenal activity, which becomes obvious from the increase in blood glucose level. These changes are illustrated in the second half of figure 4. Insulin and the conditioned stimulus produce a fall in blood glucose level. This effect

is reversed on the development of cortical inhibition, during which period the hypoglycaemic effect of insulin is also abolished.

Similar results have been obtained in experiments where the amount of catechol amines and histamine have been determined in blood, during conditioned epinephrine reflexes and the inhibition of this reflex. As is shown in FIG. 5, epinephrine , as well as the conditioned stimulus following a number of administrations of epinephrine bring about an increase in the amount of catechol amines and a decrease in that of histamine. A reversal of effects, that is to say, a fall in the amount of catechol amines and a rise in that of histamine is attained on the development of internal inhibition.

These investigations, together with many others carried out in our laboratory, indicate that there is reciprocal activity between the antagonistic systems regulating blood glucose level. This reciprocal activity was very outstanding in a number of animals met with during our experiments , carried out with the purpose of developing conditioned epinephrine hyperglycaemia. In such animals the usual hyperglycaemic effect response to epinephrine was obtained only during its first few injections. Subsequent administrations at first failed to induce any change, and then produced a fall in blood sugar level. In this connection , I should like to mention the results of experiments carried out on one dog only. As shown in fig 6,

FIG 6.

the first three intravenous administrations of 200 micrograms of epinephrine bring about a noticeable rise in blood glucose level (of about 20 mg%). During the fourth and fifth administrations,

the amount of glucose remains unchanged . Further on, during the 6th, 7th and 8th applications, epinephrine produces a substantial decrease in blood glucose level which falls down to 45 mg%. Thus a picture similar to that obtained during cortical inhibition of conditioned epinephrine hyperglycaemia was observed.

On the basis of these and many other similar results , we come to the conclusion that during a certain stage of epinephrine excitation, a depression takes place in the activity of the systems concerned with the increase of blood glucose level, of which the most important are the adrenal glands. A simultaneous activation of the antagonistic systems, of which the most important is the insular, accounts for the fall in the blood sugar level observed. This suggestion is further confirmed by the following experiments, where the systematic administrations of epinephrine lead to the cancelling of its effect or even to its reversal and in such cases, subthreshold doses of epinephrine, which during control experiments had had no effect on blood glucose level, now cause an appreciable hyperglycaemia. This is illustrated in fig 7, where 10 mg. of epinephrine are given after the cancelling the effect of 200 mg.,

FIG 7.

attained through its repeated injections. As shown in the figure a noticeable increase in blood glucose level is reached. Here we come across one of the characteristic phenomenon of the inhibitory process, called the paradoxical phase, introduced by Vedenski and confirmed by many others. This states that during inhibitory processes small doses of the stimulus cause a greater effect than larger ones.

Simultaneously with the inhibition of the cortical centres regulating the secretion and action of epinephrine, those concerned with the secretion and action of insulin are reciprocally activated. This is illustrated by the experiments shown in fig 7, where 0.5 units of insulin, which during preliminary experiments had marked no change, given upon four administrations of 200 γ of epinephrine after a reversal of its effect, caused a considerable fall during the three consequent administrations. On the fourth and fifth injections, 0.5 units of insulin had no effect on blood glucose level. These results were confirmed by many other experiments.

The reciprocal interrelationship in the activity of the antagonistic systems described above plays a significant role in the finer correlation of the mechanisms participating in the homeostatic functions of the organism, including the stability of the blood glucose level. Numerous investigations, as, carried in different laboratories, have shown that during hypoglycaemia, the secretion of epinephrine is enhanced and that of insulin depressed, while quite the contrary is observed during hyperglycaemia.

However, a more perfect regulation of the functional interrelationship between the adrenal and insular systems is realized by the cerebral cortex. This is confirmed by the investigations of Hasratian and Coworkers, and Zakharov, carried out on decorticated dogs. On decortication the organism becomes more sensitive to epinephrine and insulin administrations. Here the changes in blood glucose level last longer than in nonopera-, dogs. Similar data have been obtained by a number of other in-

vestigators as well as by us in experiments carried out on animals where cortical activity had been precluded through narcotization. This effect is most probably due to the fact that on removing the cortex thus precluding its activity, the finer correlation of the compensatory mechanism is abolished. As a result of this the action of the antagonistic mechanism for the removal of the abnormal effect, is postponed and sometimes does not even take place.

To illustrate this I will present the results of a few experiments. As I have already demonstrated in some dogs, after repeated administrations, epinephrine no more increases blood glucose level. Now, if the same amount is given to a dog under amidal or nembutal narcosis, it produces its usual hyperglycaemic effect. The results of some one such experiment are shown in fig 8 . Here the repeated intravenous injections of

FIG 8.

epinephrine produce no change in blood glucose level. The same amount, administered under narcotization produces its usual hyperglycaemic effect. Data available from the literature , and the results of our experiments, allow us to conclude that decortication and narcotization produce a state of central "denervation". if it may be so called , as a result of which reciprocal compensatory mechanisms suffer appreciably and the organism becomes more sensitive to humoral agents. Cannon's law about the increased sensitivity of denervated organs to humoral agents is explained by a number of investigators through the depression, in such organs, of these processes which conduce lead to the in-

activation of the humoral agent. For example, according to Burn and Robinson, the increased sensitivity of the denervated nictitating membrane to epinephrine is due to the decrease in monoaminoxidase activity. Armin and Grant ascribe the increased sensitivity of the denervated central artery of the rabbits ear to acetylcholine to the fall in cholinesterase activity. Went finds that in denervated structures, the formation of antimetabolites neutralizing the effect of neurohumors, is reduced.

The cortical regulation of metabolic processes may be considered a more developed stage of that same fundamental rule governing the regulation of enzymatic processes. It is well known that at a certain stage of the enzymatic process, the activity of the enzyme is suppressed the reaction being often stimulated in the opposite direction.

In connection with the cortical regulation of metabolic processes the results of some other experiments of ours are of interest. Section of the right vagus nerve, which stimulates insulin secretion, increases the sensitivity of the organism to epinephrine and lowers its sensitivity to insulin. In such an animal the effect of even small doses of epinephrine is cancelled during its third or fourth administrations.

This indicates that when insulin secretion is deprived of its nervous control, the suppression of the metabolic effect of epinephrine occurs more quickly. In this respect , the cerebral cortex plays undoubtedly an important part in the regulation of the compensatory mechanisms governing the homeostatic state of the organism.

This may be illustrated by the results obtained on dogs where the right vagus nerve had been incised. In such animals 25 mg. of epinephrine and I unit of insulin given in the course of control experiments had no effect on blood glucose level. Then followed the injections of I00 mg. of epinephrine. A noticeable hyperglycaemia was observed only on its first two administrations. The third brought no change in blood sugar level, while the fourth and fifth produced a hypoglycaemia. 25 mg. of epinephrine given during this stage induced an appreciable hyperglycaemia. This indicates the establishment of a state, characteristic of the inhibitory process, known as the paradoxical phase.

At the same time the administrations of I unit of insulin produced an appreciable lowering of blood glucose level. Thus together with the inhibition of the mechanisms taking part in producing hyperglycaemia, there was a reciprocal activation of the hypoglycaemic effect of insulin. All of this however was not observed during narcotization.

The above-mentioned facts indicate that the effect of epinephrine and insulin on blood glucose level depends on the reciprocal activity of the antagonistic systems, which are subordinated to cortical mechanisms. When one of these systems gets the upper hand the hyperglycaemic effect of epinephrine and the hypoglycaemic effect of insulin , may dissappear. During our investigations we have frequently met with the elimination of the effects of epinephrine and insulin on blood glucose level,

when they were used in cases of inhibited conditioned adrenalin and conditioned insulin hypoglycaemia. On the other hand, after repeated administrations of epinephrine, when it was replaced by insulin, during the first and sometimes even the second injections hyperglycaemia was obtained instead of the hypoglycaemia expected. Similarly, after repeated administrations of insulin, when it was replaced by epinephrine , a rise of blood glucose level was not to be seen. The hyperglycaemia characteristic of epinephrine, was noticed only on the 2nd and 3rd days of its administrations. To illustrate these facts a few examples are quoted from our investigations.

FIG 9, I0.

Fig 9 and I0 demonstrate that insulin, administered after repeated injections of epinephrine , continues to raise blood glucose level. Similarly epinephrine given after repeated daily administrations of insulin brings about hypoglycaemia.

A number of authors have failed to develop conditioned epinephrine hyperglycaemia and conditioned insulin hypoglycaemia. In the case of some animals, our repeated trials for establishing conditioned reflexes in respect to epinephrine and insulin have also been unsuccessful. On the basis of a number of facts obtained during the study of this problem, it may be concluded that the failure to develop conditioned epinephrine hyperglycaemia may be due to the depression of adrenal function with the simultaneous activation of the insular system. In some animals this condition seems to develop more quickly than in others, which probably depends on the correlative activity of the above mentioned systems and the doses of epinephrine and insulin,

applied.

The failure to develop a comditioned insulin hypoglycaemia is most probably due to the early activation of anti-insular mechanisms. With the purpose of confirming this concept of ours we have studied these effects on dogs with one adrenal removed and the other denervated. In this way we have tried to reduce the activity of the most important of the anti-insular mechanisms, .

During experiments carried out prior to the above mentioned operation, it was noticed that the conditioned stimulus, given after IO-II administrations of insulin did not induce a fall of blood glucose level.

FIG II

The results of one such experiments are illustrated in fig II.

Upon the removal of one adrenal and the denervation of another, the sensitivity towards insulin as expected, was found to be increased, while, that towards epinephrine had decreased. Then followed the systematic administrations of the usual doses of insulin which was four timed greater than its threshold doses(0.4 units). After the tenth injection of insulin the administration of the conditioned stimulus induced a pronounced hypoglycaemia. A lowering of blood glucose level was also noticed on the second, third and fourth administrations of the conditioned stimulus, after which it produced no effect on blood glucose level. Thus, the above mentioned operation by weakening one of the most powerful of the anti-insular systems, the adrenal glands, and the removal of their central nervous control led to the earlier and more permanent establishment of conditioned

insulin hypoglycaemia.

It was interesting to follow the changes in blood glucose level during the development of cortical inhibition in the above mentioned operated animals. It was observed that in these dogs, during cortical inhibition, there was no appreciable change in blood glucose level. The development of cortical inhibition, through the extinction of the conditioned insulin hypoglycaemia led, as was the case with nonoperated animals , to an increase in blood glucose level. This phenomenon had been explained through the reciprocal enhancement of anti-insular activity, especially that of the adrenal glands. In these experiments, the failure to obtain such an increase must, most probably, be explained by the weakening of the main anti-insular system. Preliminary experiments indicate that in such animals during the development of cortical inhibition no rise is noticed in blood catechol amines.

Numerous provious investigations had shown that insulin, epinephrine or any other stimulus, when given during the first few days following the development of cortical inhibition , does not manifest its characteristic effect. The same thing was observed in experiments carried out on operated animals. The results obtained on one such operated animal during the development and extinction of conditioned insulin hypoglycaemia, are given in table I.

Table I.

As shown in the table, the conditioned stimulus from its fourth administration onwards, falls to produce a hypo-

glycaemia. The subsequent administrations of the conditioned stimulus lead to the development of cortical inhibition.

On the establishment of cortical inhibition, insulin given in the same amounts as before, fails to produce any significant decrease in blood glucose level during its first, second, third and fourth administrations. Only on its fifth injection it produces a fall in blood glucose level equal to that observed in experiments proceeding cortical inhibition ----- a decrease of about 30 mg.%.

What is the reason of the absence or considerable weakening of the hypoglycaemic effect of insulin given during cortical inhibition?

One of the main mechanisms underlying the hypoglycaemic effect of insulin is its enhancement of membrane permeability to glucose, especially in muscle and fab tissue. Probably during certain functional states of the cerebral cortex especially the inhibition of conditioned insulin hypoglycaemia, membrane permeability is so changed through nervous impulses, as to produce a reversal in the effect of insulin on glucose transport. Many investigators have shown that in muscular tissue, through the action of nervous impulses, the transport of a number of substances, including the sugars, can be changed. Of particular interest are the changes in the metabolic processes of muscular tissue following its denervation. According to Axelson and Thesleff, the sensitivity of receptor sites, in the denervated muscular membrane, is increased towards acetylcholine and other agents. Such a membrane is free from cholinesterase acti-

vity and its selective permeability towards sodium and potasium is changed. Koshdoyantz and Yanson have shown that in denervated muscle the structure and properties of muscular glycogen are changed. Gerritsen has established that during the first period following denervation the glucokinase activity of muscular tissue is increased. According to Takashi, phosphorylase activity is also enhanced and is no more affected by epinephrine. A lowering in aldolase activity has also been reported.

It was interesting to study the permeability of muscular tissue to glucose following its denervation. This was studied on the gastrocnemeus muscle of frogs three days after its denervation. The results given in table 2, are the mean values of 25 tests. The uptake of

Table 2.

glucose by muscular tissue is expressed in mg. % for I gr. of fresh tissue. Incubation was carried out at 37° for 30 minutes, in Ringer's solution.

As seen from the table , the uptake of glucose by muscular tissue is about I3.5 mg. %. On the addition of insulin, this amount increases to 20 mg %. A similar rate of glucose uptake is noticed after denervation, where the addition of insulin causes a further increase in its quantity. Interesting results have been obtained on the stimulation of muscle. Here an increase in uptake of I6.3 mg % is observed. This observation is compatible with those found in the literature. Insulin, added to stimulated muscle raises glucose uptake to a level of 3I.8 mg %. A reversed effect is obtained on the stimulation of denervated muscle.

In this case, the uptake of glucose is appreciably reduced by about I0.3mg %, and what is more interesting, the addition of insulin, does not ~~in this case~~ increase glucose uptake, which remains at 8.8 mg %. These results indicate that the nervous factor has an essential role in the regulation of glucose transport brought about by insulin.

These findings are compatible with our supposition regarding the failure of obtaining a hypoglycaemic effect of insulin during the inhibition of the conditioned insulin hypoglycaemia. As will be remembered , we had already proposed, that one of the mechanisms underlying this fact may be a changed membrane permeability towards glucose , under the influence of cortical impulses. This concept was supported by other studies carried out on dogs where the utilization of glucose by skeletal muscle was determined. This was done by taking blood samples from the carotid artery and the corresponding vein in the hind limb.

Present studies emphasize the significiant role of gammoamino butyric acid (GABA) in inhibitory processes. This substance has a certain effect on membrane permeability also. In this respect we have studied the effect of GABA on the the penetration or glucose in muscular tissue.

The results of experiments carried out on the isolated rat diaphragm have shown that in muscle tissue GABA in amounts of I mg %, causes an appreciable increase in the transport of the glucose and the subsequent synthesis of glycogen.

In the abolishment of the hyperglycaemic effect of insulin on its administration in certain functional states of the brain, other anti-insular mechanisms seem to take part. Among these glucose, insulinase activity of certain tissues and the conjugation and inactivation of insulin by certain proteins of blood seem to be of some importance. These problems are being studied in our laboratory .

It has been shown that during the cortical inhibition of conditioned insulin hypoglycaemia, the elimination of the hypoglycaemic effect of insulin is accompanied by a noticeable rise in glucagon activity. Thus, for instance , 0,5 mg. of glucagon, which in control experiments had had no effect, under such conditions evokes a rise in blood glucose level of about 25 mg%. This promotion in glucagon activity is noticed also on the 2nd and 3rd days following cortical inhibition, after which it fades away. This is another fact pointing to the reciprocal activation of antagonistic mechanisms during cortical inhibition.

In this respect it is interesting to mention the results of another series of experiments , where the inactivation of a known amount of insulin following its incubation with blood was studied. It was shown that in control experiments insulin activity was halved following its incubation with blood for I at 37°C. On incubating insulin with blood samples taken during the state of cortical inhibition, when the hypoglycaemic effect of insulin was no more obtained, it was noticed that insulin activity was decreased much more, than when incubated with samples of blood taken during control experiments.

Facts , such as the abolishment of the hypoglycaemic effect of insulin during the inhibition of the conditioned insulin hypoglycaemia as well as the reversal of insulin action following the systematic administrations of epinephrine and in certain neurotic states of the organism obtained in the course of our experiments, confirm the significance of the nervous factor in the aetiology and course of diabetes. There are many instances in the literature where the origin of diabetes is connected with a nervous stress. Mirsky believes that very comparatively few cases of diabetes can be ascribed to structural lesions of the pancreas . He cites data from Bell who finds that in about 40 % of cases of diabetes no decrease in beta-granulation is observed. Some decrease is noticed in 35 %, while a complete loss of beta-granulation is observed only in 25 % of the cases. It has been reported that for the initiation of experimental diabete more than 85 % of the pancreas must be removed. These results indicate that diabetes cannot always be ascribed to pancreatic lesions, it may be of non-pncreatic aetiology, due to a decrease in the effect of insulin on tissues or to its more rapid inactivation. These processes are undoubtedly regulated by the central nervous system.